Plasma-Based Approach to Promote the Covalent Attachment of Biomolecules to Polymers

S.G. Walton and E.H. Lock, Plasma Physics Division, U.S. Naval Research Laboratory, Washington, DC; and S.H. North and C.R. Taitt, Center for Bio/Molecular Science and Engineering, U.S. Naval Research Laboratory, Washington, DC

ABSTRACT

In recent years, polymer surfaces have become increasingly popular for biomolecule attachment due to their relatively low cost and desirable bulk physico-chemical characteristics. However, the chemical inertness of some polymer surfaces poses an obstacle to their successful implementation in a broader range of biomaterials applications. We describe the use of argon plasma to generate reactive hydroxyl moieties at the surface of polystyrene microtiter plates. The plates are then selectively functionalized with silanes and crosslinkers suitable for the covalent immobilization of biomolecules. We demonstrate that this protocol supports direct, covalent immobilization of biomolecules on microtiter plates. Importantly, the biomolecules retained functionality and demonstrated attachment efficiency comparable to commercial pre-activated microtiter plates.

INTRODUCTION

Microtiter plates are effective solid-phase platforms for multiplexed, high-throughput screening and analysis of biomolecule interactions. This multi-well format, available in 6, 24, 96, 384, 1536, and even 3456 or 9600-well plates, is widely used in both industry and medical fields due to its ease of automation, high capacity for paralleled data collection, and versatile application of technologies (e.g., proteomics, functional genomics, biomolecule separation and purification).

Microtiter plates are manufactured using a variety of polymeric materials (e.g., polypropylene, polycarbonate), but polystyrene is most commonly employed because it readily adsorbs (via non-covalent interaction) proteins, has excellent optical as well as mechanical properties, and is cost-effective. However, several intrinsic properties of polystyrene also present disadvantages: poor chemical resistance, difficulty controlling surface chemistry, but most importantly, the simple physisorption can lead to protein denaturation, desorption and/or loss of biomolecule activity. [1, 2] The benefits of the covalent attachment versus physisorption of biomolecules are largely recognized. It represents a more robust approach that can ensure a more stable attachment and functional display of the biomolecule of interest. [3]

Plasmas are often used as a preparation step of polymer substrates prior to bioimmobilization as signified by numerous review articles on this subject. [4, 5] Plasmas are effective in polymer surface functionalization, polymer thin film deposition, and grafting of different functionalities onto the polymer surface. However, even though plasmas can produce the desired chemical modifications, in most cases, they also cause morphological modifications, which have been shown to affect the activity of the immobilized biomolecules. [6] Indeed, small biomolecules such as peptides, saccharides, and small proteins are highly sensitive to their microenvironment as activity is often dependent on presentation and orientation. [7, 8]. Therefore, we sought to develop a method that allows precise control over polymer surface functionalization without changes in morphology.

Electron beam-generated plasmas are ideally suited for the processing of polymers, particularly in applications where surface roughening can be problematic. When high-energy (≈ 2 keV) electron beams are used as the primary ionization source, the resultant plasmas have an inherently low electron temperature. This, of course, provides a low plasma potential and so ions impacting adjacent surfaces will do so with low kinetic energies. These characteristics depend on the working gas and operating parameters but, under typical conditions, the electron temperature does not exceed 1 eV [9] and the accompanying ion kinetic energies are only in the range of a few eV [10]. These energies are comparable to the bond strengths in typical polymers and so ion-driven surface processes – sputtering, etching, and surface roughening – are not expected to be ballistic in nature. Not surprisingly then, it has been demonstrated that electron beam generated plasmas can be used to modify surface chemistries without removing much material or significantly changing the surface morphology. [11-13] It is with this in mind, that we have investigated the use of these plasmas as the first, in a multi-step protocol for the immobilization of both large and small biomolecules on polystyrene microtiter plates.

© **2011 Society of Vacuum Coaters** 505/856-7188

54th Annual Technical Conference Proceedings, Chicago, IL April 16–21, 2011 ISSN 0737-5921

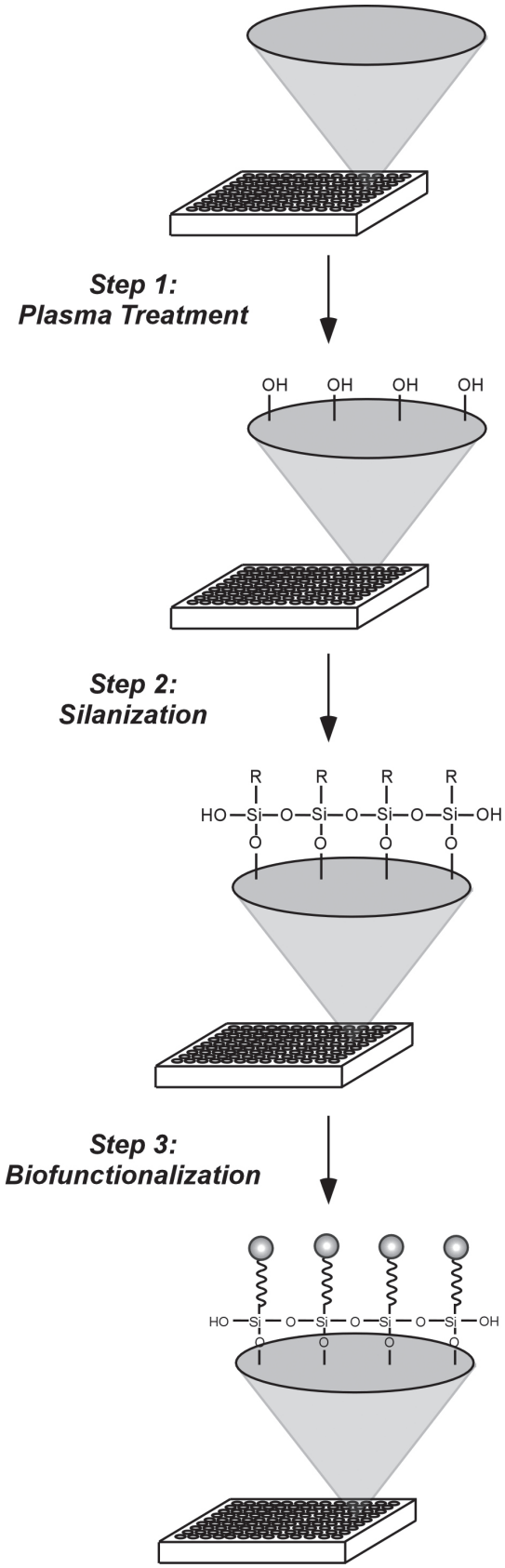

Figure 1: The 3-step method for the treatment of microtiter plates that leads to covalent bioimmobilization.

METHODOLOGY, RESULTS, AND DISCUSSION

The method for the surface modification of polystyrene microtiter plates, shown in Figure 1, has been detailed in [14]. Broadly, it consists of three distinct but dependent steps, comprising both dry and wet chemical processing, to yield a versatile, covalent immobilization platform for biomedical and biological applications: (1) plasma-based introduction of surface hydroxyl groups using apparatus described in [15], (2) silane monolayer assembly using organo-functional silanes, and finally, (3) covalent coupling of the biomolecule of interest using crosslinkers of variable functionality and spacer length for user-defined surface characteristics.

A scheme for the immobilization of protein (e.g., antibody) is depicted in Figure 2. The generation of hydroxyl groups on the surface was accomplished by first exposing the plates to pulsed, electron beam generated plasma produced in argon and then exposing the plates to air. The OH-enriched, plasma-modified surfaces were then treated with (3-mercaptopropyl) triethoxysilane (MPTES) to yield surfaces functionalized with pendant thiol moieties. The silanized surfaces were treated with a heterobifunctional crosslinker (GMBS), followed by the immobilization of antibodies.

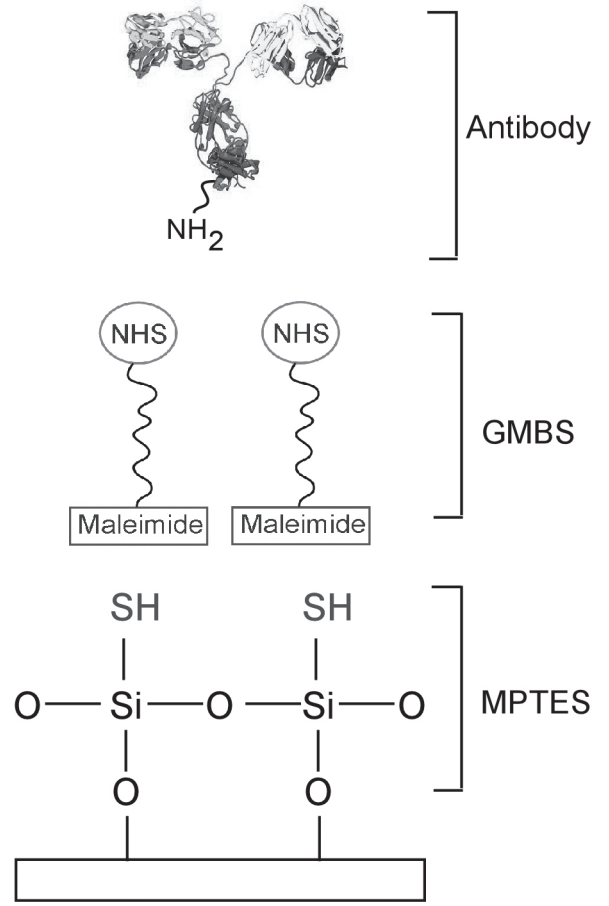

Figure 2: Immobilization scheme using mercaptosilane (MPTES) and GMBS crosslinker.

Efficiency of bio-immobilization was determined by measuring fluorescence of attached fluorophore-labeled antibodies (Cy3-IgG). The results in Figure 3 for antibody immobilization, clearly show plasma treatment is critical. However, choosing the correct plasma operating parameters is also important; under- or over-treatment will not yield high immobilization efficiencies. Importantly, the highest efficiencies were linked to the highest oxygen concentrations at the surface. [16]

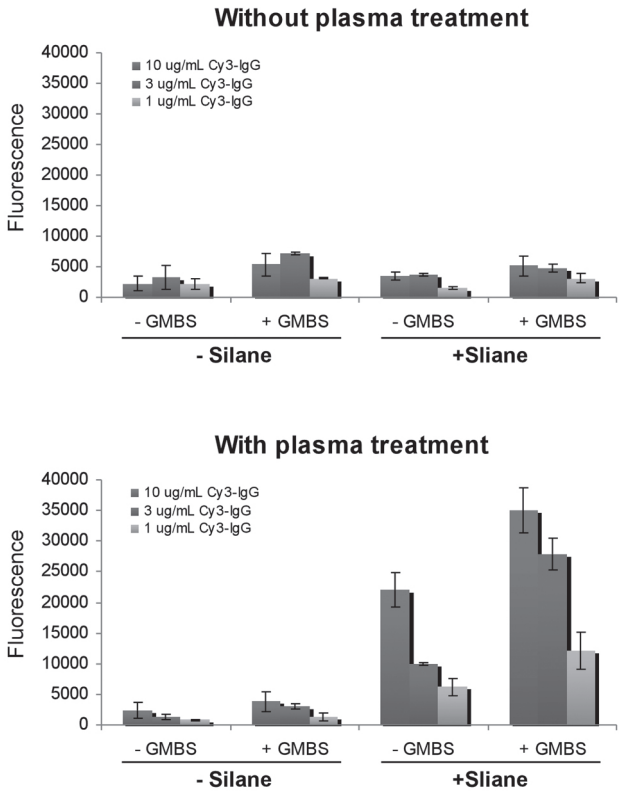

Figure 3: Fluorescent intensities of Cy3-IgG binding on plasma-treated (bottom) and untreated (top) polystyrene microtiter plates using the scheme depicted in Figure 2. Note, the – and + refer to the absence or presence of silane/crosslinker.

The issues of biomolecule denaturation, desorption and/or loss of biomolecule activity that could occur when biomolecules are immobilized through physisorption have been recognized and not surprisingly, a number of commercial microtiter plate are available in pre-activated form for covalent attachment of biomolecules. We compared the efficacy of biomolecule binding and retention using the immobilization scheme described above with those of commercial microtiter plates, and found the bioimmobilization efficiency to be superior. Those results are shown in Figure 4.

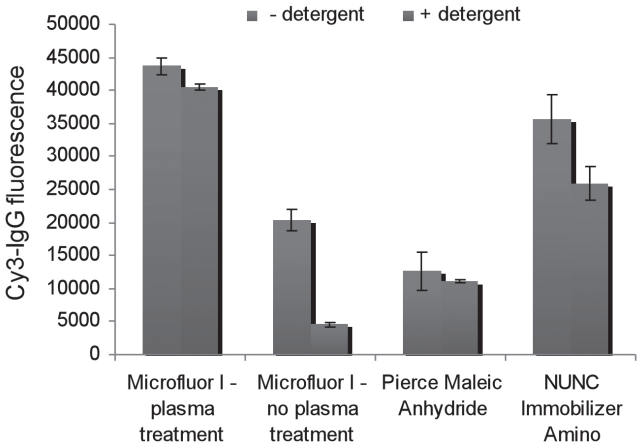

Figure 4: Fluorescent intensities of Cy3-IgG (10 µg/mL) immobilized on plasma-treated and untreated plates Microfluor I (covalent immobilization following Fig. 2), Microfluor I (physisorption), Pierce Reactibind Maleic Anhydride and NUNC Immobilizer Amino (covalent immobilization according to manufacturers' instructions); blue bars = no detergent wash, red bars = detergent wash.

It is important to note that the results were not limited to the combination of silane and crosslinkers described above, suggesting the plasma treated plates have a significant potential for tailored attachment chemistries, which is not typically found in commercially available plates.[14]

CONCLUSION

A protocol for bioimmobilization on polystyrene microtiter plates that combines plasma functionalization with wet chemical processing was described. The use of plasmas to decorate the polymer surface with -OH moieties was shown to be a critical step in the bioimmobilization protocol. This step provided for a versatile, covalent immobilization scheme with an immobilization efficiency that surpassed those available on both untreated and commercially available microtiter plates.

ACKNOWLEDGEMENTS

This work was funded by the Joint Science & Technology Office for Chemical & Biological Defense/Defense Threat Reduction Agency and the Office of Naval Research.

REFERENCES

1. D.E., Rebeski, E.M. Winger, Y.-K. Shin, M. Lelenta, M.M. Robinson, R. Varecka, J.R. Crowther, J. Immunol. Methods 226, 85 (1999).

2. J.E. Butler, Methods 22, 4 (2000).

3. J.M. Goddard, J.H. Hotchkiss, Prog. Polymer Sci. 32, 698 (2007).

4. T. Desmet, R. Morent, N.D. Geyter, C. Leys, E. Schacht, and P. Dubruel,Biomacromolecules 10, 2351 (2009).

5. K.S. Siow, L. Britcher, S. Kumar, and H.J. Griesser, Plasma Process. Polym. 3, 392 (2006).

6. J.M. Goddard and J.H. Hotchkiss, Progress Polymer Sci. 32, 698 (2007).

7. N.V. Kulagina, K.M. Shaffer, F.S. Ligler, and C.R. Taitt, Sens. Actuators B 121, 150 (2007).

8. K. Inamori, M. Kyo, K. Matsukawa, Y. Inoue, T. Sonoda,K. Tatematsu, K. Tanizawa, T. Mori, and Y. Katayama, Anal. Chem. 80, 643 (2008).

9. E.H. Lock, R.F. Fernsler, and S.G. Walton, Plasma Sources Sci. Technol. 17, 025009 (2008).

10. S.G. Walton, C. Muratore, D. Leonhardt, R.F. Fernsler, D.D. Blackwell, and R.A. Meger, Surf. Coat. Technol., 186 (2), 40 (2004).

11. E.H. Lock, S.G. Walton, and R.F. Fernsler, Plasma Proc. and Poly. 6, 200800122 (2009).

12. S.G. Walton, E.H. Lock, A. Ni , M. Baraket, R.F. Fernsler, D.D. Pappas, K.E. Strawhecker, and A.A. Bujanda, J. Appl. Polym. Sci. 117, 3515 (2010).

13. E.H. Lock, D.Y. Petrovykh, P. Mack, T. Carney, R.G. White, S.G. Walton and R. F. Fernsler, Langmuir 26(11) 8857 (2010).

14. S.H. North, E.H. Lock, C.J. Cooper, J.B. Franek, C.R. Taitt, and S.G. Walton, ACS Applied Materials & Interfaces, 2(10), 2884 (2010).

15. S.G. Walton, E.H. Lock, D. Leonhardt, and R.F. Fernsler, *50th Annual Technical Conference Proceedings of the Society of Vacuum Coaters*, pp 125-128, 2007.

16. E. H. Lock, S. H. North, J. Wojciechowski, C. R. Taitt, and S. G. Walton, Technological Innovations in Detection and Sensing of Chemical-Biological-Radiological-Nuclear Threats and Ecological Terrorism, NATO Science for Peace and Security Series - A: Chemistry and Biology, edited by Ashok Vaseashta (Springer, Netherlands, 2011), in press.